DNA Damage Induced in Cultured Human Alveolar (L-132) Cells by Exposure to Dimethylarsinic Acid

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Gene damage in cultured human alveolar (L-132) cells induced by exposure to dimethylarsinic acid (DMAA), a major metabolite of inorganic arsenics in mammals, was studied. DNA single-strand breaks and DNA-protein cross-links were induced by the treatment of L-132 cells with 10 mM DMAA. These kinds of damage appeared at 8 hr after start of exposure to DMAA. As regards DNA-protein cross-links, the DNA was found to bind not only to core histone proteins but also linker histone (H1) and nonhistone proteins. Furthermore, the cross-links were formed by the binding to serine or threonine residues of H1 or nonhistone proteins through phosphate moieties of the DNA. The induction of the alkali-labile sites in DNA in DMAA-treated L-132 cells was observed prior to that of DNA single-strand breaks and DNA-protein cross-links. As one of the alkali-labile sites in DNA, we estimated apurinic/apyrimidinic (AP) sites in DNA. The present study suggests that the DNA single-strand breaks and DNA-protein cross-links induced by the treatment of L-132 cells with DMAA occurred via the formation of AP sites in the DNA and that the DNA-protein cross-links were produced by a Schiff-base reaction between amino groups of nuclear proteins and aldehyde groups of AP sites in the DNA and the DNA single-strand breaks, by a β-elimination reaction on AP sites in the DNA. — Environ Health Perspect 102(Suppl 3):285–288 (1994).

Key words: dimethylarsinic acid, DNA damage, L-132 cells, cross-link, DNA strand breaks, apurinic/apyrimidinic (AP) site, active oxygen, free radical, arsenic peroxyl radical, inorganic arsenics

Introduction

Although inorganic arsenics are epidemiologically proved to be carcinogenic for lung and skin (*1,2*), experimental studies so far have not succeeded in evidencing their carcinogenicity. On the other hand, among a number of investigations on the genotoxicity of inorganic arsenics, some data (*3–6*) have suggested that inorganic arsenics are not direct-acting mutagens, but act as co-mutagens with other carcinogens, e.g., ultraviolet and alkylating agents, through modification of the DNA repair process. In fact, Li and Rossman reported that the activity of DNA ligase II was suppressed by arsenite (*7*). However, many more studies will be required to solve the genotoxic action of inorganic arsenics.

We have investigated genotoxic effects of arsenics (*8–16*) and, of these arsenics, we paid special attention to dimethylarsinic acid (DMAA), a major metabolite of inorganic arsenics in mammals. We found that DMAA administration induced lung-specific DNA damage in mice (*10,11*), and further, estimated that the DNA damage might be due to the dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\cdot$] and active oxygens produced in further metabolic processing of the DMAA (*12*).

This article describes the gene damage induced by exposure of L-132 cells, an established human cell line of alveolar type-II cells, to DMAA. We found that DNA single-strand breaks (*15*) and DNA-protein crosslinks (*16*) in L-132 cells were induced by treatment with DMAA, as observed in *in vivo* experiments (*10,11*), and assumed that the damage might be induced via the formation of apurinic/apyrimidinic (AP) sites in DNA.

Materials and Methods

L-132 cells, an established human embryonic cell line of alveolar epithelial cells (*17*), were obtained from ICN Biochemicals Inc. (Costa Mesa, CA). The cells were cultured in Eagle's minimal essential medium (Nissui Pharmaceutical Co. Ltd., Tokyo, Japan) supplemented with 10% fetal calf serum (JRH Biosciences, Lenexa, KS). The cells were incubated with 10 mM DMAA in the medium in a humidified atmosphere of 5% CO_2 at 37°C. [Methyl-^{3}H]thymidine ([^{3}H]thymidine, 2.92 TBq/mmole) and L-[U-^{14}C]lysine·HCl ([^{14}C]lysine, 9.99 GBq/mmole) were purchased from ICN Biomedicals Inc. (Costa Mesa, CA). For labeling of DNA and nuclear proteins, the cells were grown for 20 to 24 hr in the medium containing 37 kBq/mL of [^{3}H] thymidine and [^{14}C] lysine, respectively.

The alkaline elution method established by Kohn et al. (*18*) was used to measure DNA single-strand breaks; and alkaline agarose gel electrophoresis (*19*), was used to measure alkali-labile sites in DNA. To detect AP sites in DNA, DNA was isolated by phenol extraction from L-132 cells. The DNA was treated with a restriction enzyme (Kpn I) and then subjected to alkaline agarose gel electrophoresis. The formation of DNA-protein cross-links was determined according to the filter binding assay described by Strniste and Rall (*20*). For separation of DNA-nuclear protein complexes, chromatin prepared from L-132 cells according to the method

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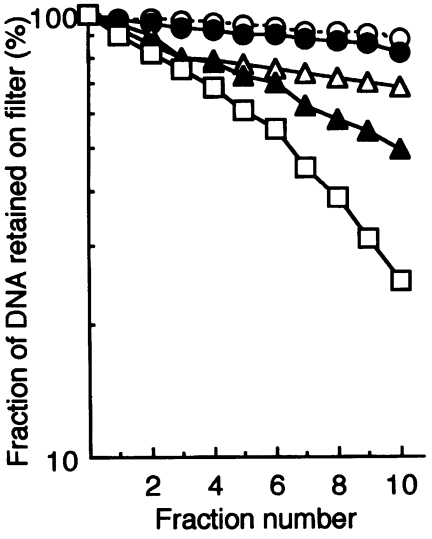

Figure 1. DNA single-strand breaks induced by exposure of L-132 cells to DMAA. L-132 cells were treated with 10 mM DMAA for 0 hr (○), 6 hr (●), 8 hr (△), 10 hr (▲), 12 hr (□) in Eagle's MEM supplemented with 10% fetal calf serum.

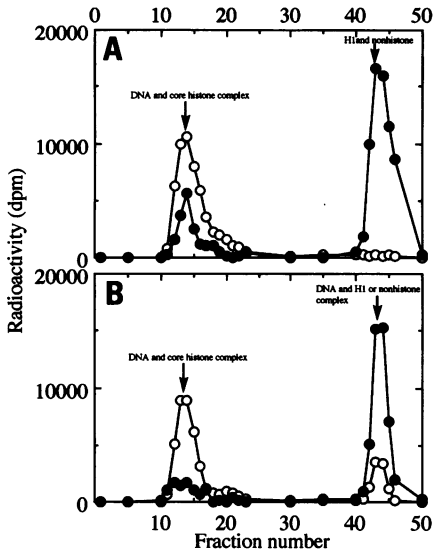

Figure 2. Chromatography of the chromatin from L-132 cells after exposure to DMAA. L-132 cells prelabeled with [^{3}H] thymidine and [^{14}C]lysine were left untreated (A) or treated (B) with 10 mM DMAA for 12 hr in Eagle's MEM supplemented with 10% fetal calf serum. The soluble chromatin from the cells was chromatographed on Sepharose 4B. ^{3}H-radioactivity (○) of DNA and ^{14}C-radioactivity (●) of nuclear proteins in each fraction were measured in a liquid scintillation counter. The chromatographic conditions were as follows: column size, 1.6 x 20 cm; eluent, 0.45 M NaCl/10 mM sodium bisulfite/10 mM EDTA/25 mM Tris-HCl (pH7.0); fraction size, 2 ml; flow rate, 0.2 ml/min.

of Haung and Haung (*21*) was chromatographed on Sepharose 4B (*22*).

Induction of DNA Single-Strand Breaks and DNA-Protein Crosslinks

To confirm if DNA single-strand breaks found in mouse lung *in vivo* can be observed also in an *in vitro* cell culture system, we determined the strand breaks in L-132 cells exposed to DMAA by the alkaline elution method (*15*). When L-132 cells were incubated in medium containing DMAA at concentrations of 5, 7.5, and 10 mM for 12 hr, concentration-dependent single-strand breaks occurred. Furthermore, as shown in Figure 1, in the case of exposure to 10 mM DMAA, the induction of the single-strand breaks was dependent on the duration of incubation with the DMAA. The breaks were not detected up to 6 hr incubation but were observed remarkably at 8 to 12 hr after start of DMAA exposure. To evaluate the recovery from the breaks, we first exposed L-132 cells to 10 mM DMAA for 12 hr and then transferred them to DMAA-free medium; thereafter, the degree of the single-strand breaks was determined by the alkaline elution assay. The recovery proceeded time-dependently; the breaks were partially recovered at 2 to 4 hr and fully at 6 hr after transfer into the DMAA-free medium.

On the other hand, for the measurement of DNA-protein cross-links, the filter binding assay was performed (*16*). As shown in Table 1, the amount of DNA retained on the filter was not changed up to 6 hr but increased by 12 hr after exposure to DMAA. These results indicate that DNA single-strand breaks and DNA-protein cross-links occur similarly in mouse lung *in vivo* (*11,16*) and in L-132 cells *in vitro* (*15,16*), suggesting that the present *in vitro* system is useful to analyze the DNA damage observed in the *in vivo* experiments.

Characterization of DNA-Protein Cross-links

To characterize the type of the cross-links induced by the DMAA treatment, the chromatin, with its DNA and nuclear proteins prelabeled with [^{3}H]thymidine and [^{14}C]lysine, respectively, prepared from L-132 cells, was sheared in a buffer containing 0.45 M NaCl using a Polytron homogenizer. Under this condition, linker histone (H1) and nonhistone proteins are dissociated from DNA but core histone proteins are not (*22*). The soluble chromatin sample was separated on Sepharose 4B column chromatography, and each fraction was measured for ^{3}H-radioactivity of DNA and ^{14}C-radioactivity of protein (*16*). The control sample gave two peaks (Figure 2*A*); one was DNA-core histone complex in the void volume and the other, free nuclear proteins (H1 and nonhistone proteins). The sample from the cells exposed to 10 mM DMAA for 12 hr, on the other hand, showed a marked change; some ^{3}H-radioactivity appeared in the fraction of free nuclear proteins, indicating the presence of DNA fragments associated with the proteins (Figure 2*B*). This result indicates that the formation of DNA cross-links occurred not only with core histone, but also with H1 or nonhistone proteins. The cross-link between DNA and nuclear proteins was dissociated by the treatment with 6 M guanidine hydrochloride (*16*). As it is known that the bonds of phosphoserine and phosphothreonine are labile to 6 M guanidine hydrochloride (*23*), the cross-link formation is thus very likely due to the binding between phosphate moieties of DNA and serine or threonine residues of H1 histone or nonhistone proteins (*16*).

Table 1. DNA-protein cross-links induced by exposure of L-132 cells to DMAA.[a]

Incubation time, hr	Viability, %	DNA retained on filter, %
0	93.8	5.7
6	85.5	5.4
12	92.3	23.7

[a]Cells were treated with 10mM DMAA in the medium. DNA-protein cross-links were detected by the filter-binding assay.

Formation of AP Sites in DNA in DMAA-Treated L-132 Cells

To clarify DNA damage induced at the early stage after exposure to DMAA, we used alkaline agarose gel electrophoresis to examine whether the exposure to DMAA of L-132 cells formed alkali-labile sites in DNA. As shown in Figure 4, DNA in the cells exposed to DMAA for 6 hr underwent a greater degree of alkaline hydrolysis than control DNA. This result indicates that, by exposure of L-132 cells to 10 mM DMAA, alkali-labile sites were formed in the DNA prior to the induction of DNA single-strand breaks and DNA-protein cross-links, suggesting the formation of AP sites in DNA at an early stage of DMAA exposure. On the other hand, Liuzzi and Talpaert-Borle (*24*) reported that methoxyamine bound to aldehyde groups of AP sites in DNA and that the methoxyamine-bound AP sites were more resistant to the hydrolysis than nonbound AP sites under alkaline conditions. In fact, when L-132 cells were exposed to DMAA for 6 hr, the DNA preteated with methoxyamine became alkali-resistant (Figure 3). These results indicate that DMAA-treated L-132 cells

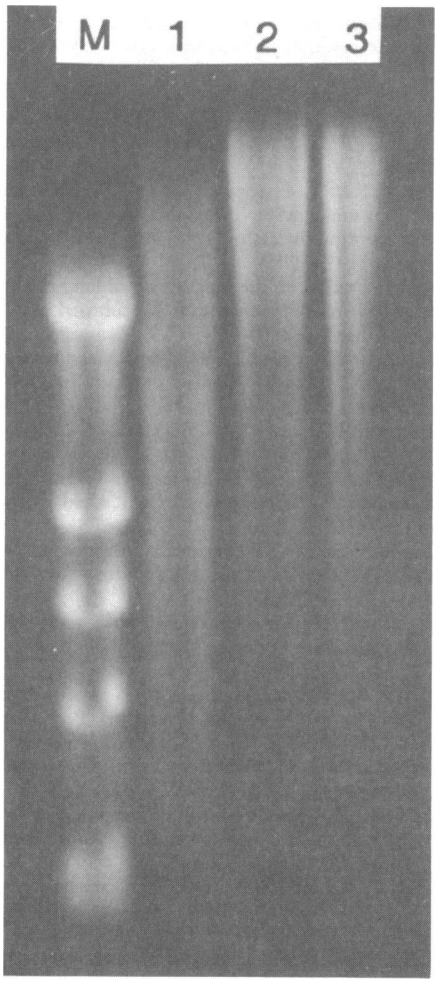

Figure 3. Alkaline agarose gel electrophoresis of DNA from DMAA-treated L-132 cells. L-132 cells were treated with 10 mM DMAA for 6 hr in Eagle's MEM supplemented with 10 % fetal calf serum. DNA was isolated by phenol extraction from the cells and then treated with restriction emzyme (Kpn I). The digested DNA in alkaline loading buffer (50 mM NaCl/1 mM EDTA/2.5% Ficoll/0.025% bromocresol green) was analyzed by 0.5% agarose gel electrophoresis in 30 mM NaOH/1 mM EDTA. The detection of DNA was performed by ethidium bromide staining. Lane M, I-Hind III digest; lane 1, DNA from L-132 cells treated with 10 mM DMAA for 6 hr; lane 2, DNA from L-132 cells treated with 10 mM DMAA for 6 hr and 5 mM methoxyamine; lane 3, DNA from nontreated cells (control).

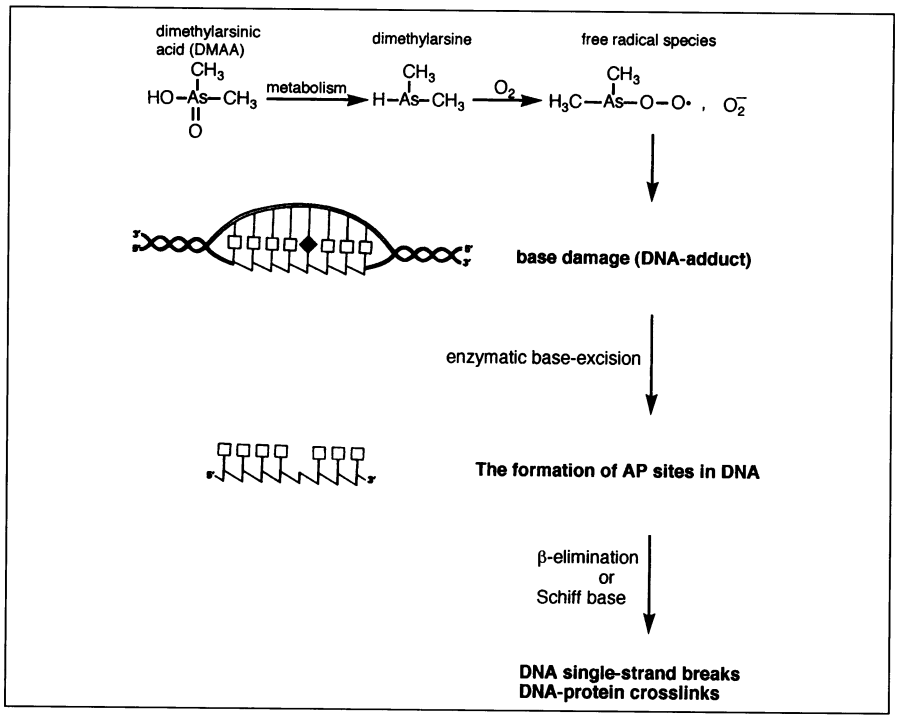

Figure 4. Proposed mechanism of DNA damage by the free radicals produced in the metabolic processing of DMAA.

formed AP sites in the DNA prior to the induction of DNA strand scissions (*15*) and DNA-protein cross-links (*16*).

Discussion

Based on the present study, we propose a possible pathway of DNA damage induced by dimethylarsenics, as schematically shown in Figure 4. We had assumed that lung-specific DNA damage induced by DMAA administration in mice might be attributed to the dimethylarsenic peroxyl radical [$(CH_3)_2AsOO\cdot$] and active oxygens produced in the metabolic processing of DMAA (*12*). We even speculated that DNA single-strand breaks and DNA-protein cross-links were directly caused by these radicals, as in the case of ionizing radiation (*11*). However, the results obtained from the present study may not fully support our previous speculation. Here we assume that DNA single-strand breaks and DNA-protein cross-links in L-132 cells exposed to DMAA may be indirectly caused by these radicals via the formation of AP sites in DNA. As regards the induction mechanism of DNA damage, we estimated that the cross-link formation occurred by a Schiff-base reaction between amino groups of nuclear proteins, particularly of H1 and nonhistone proteins, and aldehyde groups of AP sites in DNA and that the induction of DNA single-strand breaks occurred after a β-elimination reaction at AP sites in the DNA. On the other hand, some of the base damage induced by exposure to alkylating agents such as *N*-methyl-*N*-nitrosourea and dimethylsulfate is known to be repaired through the excision of damaged bases by the action of DNA glycosylase, which hydrolyses *N*-glycosyl bonds to form AP sites in DNA (*25*). The formation of AP sites is considered one of the important processes of excision repair of not only alkylated bases, but also a variety of DNA adducts. We therefore speculate that the exposure of L-132 cells to DMAA might cause enzymatic formation of AP sites in DNA via the production of base damage such as DNA-adduct formation (*15*).

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